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研究課題名	Ferritin nanocaged doxorubicin for targeted hepatocellular carcinoma therapy	
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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

During the past year, we further optimized the method of ferritin loading doxorubicin. We int end to increase the doxorubicin loading efficiency of ferritin nanocage to further enhance the effic acy of HFn-Dox in hepatocellular carcinoma targeted therapy.

In the previous work, based on the unique shell-core structure of human natural ferritin, a 24 -mer ferritin nanoshell structure with a outer shell diameter of 12 nm and an inner cavity of 8 n m was biomimetically synthesized. Using a urea-based depolymerization/repolymerization of the fer ritin nanocage, doxorubicin (about 33 doxorubicin molecules) is loaded in its cavity. Relying on the natural tumor targeting ability of ferritin, ferritin drugs can efficiently target and kill tumors. Ho wever, the inefficient drug loading method has plagued the clinical transformation of ferritin drugs.

Recently, we found that through direct incubation, doxorubicin (Dox) molecules can enter the cavity of human heavy chain ferritin (HFn). The flexible region composed of 89-92 amino acid re sidues is related to the loading channel of Dox. Further experiments have proved that the tempera ture can control the "switch" state of the drug loading channel. Under the condition of elevated te mperature, the amino acid residues at positions 89-92 swing away from the position of the dual a xis. At this time, the channel is in the "open" state, and the drug enters into the cavity of ferritin nanocage.

Based on this finding, high-efficiency loading of doxorubicin (approximately 100 doxorubicin molecules) can be achieved by co-incubating human heavy chain ferritin (HFn) with the chemother apeutic drug doxorubicin hydrochloride at 60°C for 4 hours. Compared with the previous loading method based on depolymerization/repolymerization of ferritin, this loading method does not need t o add denaturant to destroy the structure of ferritin, and significantly improves the drug loading c apacity and carrier recovery rate.

In the *in vivo* anti-tumor experiment, Dox-loaded HFn was targeted and enriched in the tumo r site of tumor-bearing mice, resulting in the apoptosis of a large number of tumor cells, and the tumor growth could be significantly inhibited only by the second administration. Under the premis e of the same dosage, compared with the clinical drug liposomal doxorubicin (Doxil), tumor-bearin g mice still had an 80% survival rate on the 40th day after HFn-Dox treatment, and the body we ight change was less than 5%. In addition, compared with free Dox, the maximum tolerated dose of HFn-Dox in mice increased by 4 times, which significantly reduced the toxic side effects of D

ox *in vivo*. It efficiently delivers Dox to hepatocellular carcinoma tissue without damaging normal tissue, and has low immunogenicity and good biological safety.

In January 2023, professor Xiyun Yan visited the Institute of Medical Sciences, the University of Tokyo and stayed in Japan for 15 days. During this period, she had in-depth talks with Mr. Inoue, Mr. Kawaguchi, Mr. Osada, Mr. Yoshihiro ITO and others to discuss future scientific research cooperation in the fields of life science and medicine.